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How to cite:

Lipson, David A.; Jha, Mony; Raab, Theodore K. and Oechel, Walter C. (2010). Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil. *Journal of Geophysical Research*, 115(G4), article no. G00I06.

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Version: Version of Record

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<http://dx.doi.org/doi:10.1029/2009JG001147>

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Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil

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Received 2 September 2009; revised 29 June 2010; accepted 24 August 2010; published 18 December 2010.

[1] Arctic peat soils contain vast reserves of organic C and are largely anaerobic. However, anaerobic respiration, particularly the role of Fe(III) and humic substances as electron acceptors, is not well understood in such ecosystems. We investigated these processes in a drained thaw lake basin on the Arctic coastal plain near Barrow, Alaska. We measured concentrations of soluble Fe and other potential electron acceptors, described the microbial community, and performed experiments in the laboratory and field to measure net rates of Fe(III) reduction and the relationship of this process to C cycling. In most areas within the basin, aerobic conditions existed only in the upper few centimeters of soil, though oxygen penetrated deeper in raised areas, such as rims of ice wedge polygons. Concentrations of nitrate and sulfate in soil pore water were low or negligible. Soil pore water contained surprisingly high concentrations of Fe(II) and Fe(III), in the range of hundreds of μM , suggesting the presence of organic chelators. The solid phase contained substantial amounts of iron minerals, with a progressively reduced oxidation state throughout the growing season. The most abundant 16S rRNA sequence in our gene survey was closely related to the Fe(III)-reducing bacterium, *Rhodoferrax ferrireducens*, and other sequences closely related to Fe-transforming bacteria were found. Field and laboratory incubations with soluble Fe(III) and the quinonic compound, AQDS (a common humic analog), stimulated respiration and verified that Fe(III) reduction occurs in these soils. We conclude that reduction of Fe(III) and humic substances are major metabolic pathways in this ecosystem.

Citation: Lipson, D. A., M. Jha, T. K. Raab, and W. C. Oechel (2010), Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil, *J. Geophys. Res.*, 115, G00I06, doi:10.1029/2009JG001147.

1. Introduction

[2] Arctic soils represent a huge C pool with a large potential for loss, given the relatively rapid effects of climate change at high latitudes [Dutta *et al.*, 2006; Lawrence and Slater, 2005; Oechel *et al.*, 1993; Pastor *et al.*, 2003; Schuur *et al.*, 2008]. Therefore, there continues to be interest in understanding the C cycle in Arctic ecosystems. In particular, there is a gap in our understanding of the contributions of and controls over anaerobic respiration. Arctic peat soils are generally water-logged, as drainage is blocked by impermeable permafrost, evapotranspiration rates are low, and peat has extremely high water-holding capacity. It is therefore assumed that respiration is strongly limited by oxygen availability and that drying, as may likely be induced by climate change, will lead to large increases in CO_2 fluxes and decreases in CH_4 flux [Clymo, 1984; Freeman *et al.*, 1993; Knorr and Blodau, 2009; Silvola *et al.*, 1996]. How-

ever, experimental reductions in the water table of peatlands do not always have the expected effect on soil respiration [Strack and Waddington, 2007; Updegraff *et al.*, 2001]. While the reasons for this are complex [Knorr and Blodau, 2009], a deeper understanding of anaerobic respiration is warranted in these systems.

[3] The importance of ferric iron (Fe(III)) and humic substances as electron (e^-) acceptors in anaerobic respiration has become well established [Cervantes *et al.*, 2002; Lovley *et al.*, 1996; Nevin and Lovley, 2002]. These e^- acceptors are unique in that they are generally found mostly in insoluble, extracellular forms, creating the need for microbes to transport e^- to external acceptors [Stams *et al.*, 2006]. Such exocellular e^- transport has significant ecological importance, as it impacts the rates of CO_2 and CH_4 flux in anaerobic environments. For example, it has been shown that the presence of Fe(III) or humic e^- carriers can inhibit methanogenesis by providing more thermodynamically favorable metabolic pathways [Bond and Lovley, 2002; Cervantes *et al.*, 2000; Teh *et al.*, 2008]. However, the importance of these processes has only just begun to be integrated into understandings of C cycles in natural ecosystems [Chacon *et al.*, 2006; Heitmann *et al.*, 2007; Roden and Wetzel, 1996; Teh *et al.*, 2008].

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[4] Especially little is known about the potential role of exocellular electron transport in Arctic peat soils. In a study of the electrical conductivity properties of a peat soil, it was found that the electric double layer (responsible for the conductivity associated with surfaces) was far more significant in this organic soil than previously observed in mineral soils [Comas and Slater, 2004]. Hence, soil surfaces in organic soils may be capable of effectively conducting electricity away from anaerobically respiring bacteria. An analysis of the e^- balance in a rice paddy soil revealed that not all e^- could be accounted for by changes in CO_2 , CH_4 , and H_2 , indicating a potentially important role for humics as e^- acceptors in these organic-rich anaerobic soils [Yao and Conrad, 2000]. It was observed in a northern bog that a large portion of anaerobic respiration could not be accounted for by methanogenesis or aerobic respiration and that dissolved organic matter was a potentially important e^- acceptor in that ecosystem [Heitmann et al., 2007]. Terrestrially derived dissolved organic matter from Toolik Lake, Alaska, contains redox-active quinone groups, which appear to be ubiquitous in humic substances from a wide range of environments [Cory and McKnight, 2005; Fimmen et al., 2007]. Additionally, bacterial enrichment cultures capable of reducing insoluble iron oxides at low temperatures were obtained from Arctic permafrost [Zhang et al., 1999]. These studies indicate that exocellular e^- transfer to iron and/or humic substances may also play a significant role in respiration in Arctic peatlands and could contribute substantially to the C cycle in these ecosystems. However, the role of Fe and humic substances as e^- acceptors has not yet been integrated into an understanding of C cycling in Arctic soils.

[5] Peat soils in drained thaw lake basins (DTLB) are likely to support a major role for exocellular e^- transfer. DTLB cover about 50% of the Arctic coastal plain [Hinkel et al., 2003; Hinkel et al., 2005]. They are rich in C, having thick organic layers [Bockheim et al., 2004], and are water saturated most of the growing season [Hinkel et al., 2001]. Being waterlogged and without access to sulfate from seawater, they are likely to be generally exhausted of conventional e^- acceptors and are accordingly known to be sources of methane [Rhew et al., 2007; Zona et al., 2009]. In particular, young-medium aged DTLB (<300 years old) have organic layers of about 15 cm or less in thickness and therefore about 15 cm or more of underlying mineral material lies within the active layer [Hinkel et al., 2003], presumably increasing Fe availability. Therefore, our goal in this study was to investigate the potential importance of Fe (III) and humic substances in anaerobic respiration in peat soils within a medium aged DTLB on the Arctic coastal plain near Barrow, Alaska. We describe the availability of e^- acceptors in soil pore water, microbial community composition, and experiments performed in the laboratory and field to estimate rates of Fe transformation and their relationship to C cycling.

2. Methods

2.1. Site Description

[6] This study took place in and around the Biocomplexity Experiment on the Arctic coastal plain near Barrow,

Alaska. The study area is a DTLB, estimated to be 50–300 years old [Hinkel et al., 2003]. The organic layer is about 12–15 cm thick, overlying a silty, marine-derived mineral layer. Maximum thaw depth is about 35 cm. Vegetation is dominated by mosses, *Carex aquatilis*, *Eriophorum vaginatum*, and *Dupontia fisheri* [Zona et al., 2009]. Details of this site have been described elsewhere [Brown et al., 1980; Hinkel et al., 2001; Zona et al., 2009]. The majority of the DTLB is saturated most of the summer, but some ice wedge polygonization has occurred, causing some areas to be elevated and therefore relatively aerobic and dry. In areas where polygonization is weak, there are also raised hummocks of sphagnum with similar properties. Mean soil pH was 5.03, with lower values in topographically high areas such as polygon rims or sphagnum hummocks (minimum pH of 3.75), and higher values in low areas of the landscape, such as polygon centers (maximum pH of 5.75). Mean electrical conductivity of the soil was 361 $\mu S/cm$, with minimum values near zero in topographically high areas and maximum values of 624 $\mu S/cm$ in the rest of the landscape. The study area is roughly elliptical, approximately 1.45 km in length and 0.3 km wide. The majority of soil and water samples used in this study were collected along three 300 m transects that traversed the basin at the south, central, and north locations [Zona et al., 2009].

2.2. Analysis of Soil Pore Water, O_2 Profiles, and Oxidation-Reduction Potential

[7] Soil pore water was sampled using 10 cm long polymer-coated soil pore water samplers (Rhizon, Ben Meadows, Janesville, WI) inserted into the upper 10 cm of soil and collected using sterile vacutainers attached by hypodermic needles. Soluble Fe in soil pore water was analyzed colorimetrically using 1,10-*o*-phenanthroline [Analytical Methods Committee, 1978], which reacts with Fe(II). Total soluble Fe was determined by adding ascorbic acid, and Fe (III) was determined by difference [Knorr and Blodau, 2009; Tamura et al., 1974]. $FeSO_4$ solution in 10 mM HCl was used as a standard. On a subset of samples, Fe and other elements were measured by inductively coupled plasma spectroscopy (ICP) at the SDSU Ecology Instrument Facility, using an Optima 4300 DV (Perkin-Elmer, Shelton, CT). Nitrate was measured using an automated ion analyzer (LACHAT, Milwaukee, WI), and nitrite was measured colorimetrically [Bremner and Mulvaney, 1982] using a plate reader (SpectraMax190, Molecular Devices, Sunnyvale, CA). Sulfate was assayed using a turbidometric method with barium chloride [Lundquist et al., 1980]. Because of the dark color of the soil water samples, it was necessary to subtract the absorbance of untreated samples. Using this method, our lowest standard (10 μM sulfate) was easily differentiable from blanks. Dissolved organic C (DOC) in soil pore water was measured colorimetrically using a method based on reaction with Mn(III) using a glucose standard [Bartlett and Ross, 1988]. Ultraviolet (UV) absorbance of pore water was measured at 260 nm (A_{260}) to estimate the aromaticity of the DOC. A_{260} readings were standardized using the aromatic amino acid, tyrosine. Fine scale O_2 profiles were measured using a microelectrode protected by a hypodermic needle, attached to a portable picoammeter (Unisense, Denmark). A zero reading was

obtained using a 0.1 M sodium hydroxide/0.1 M sodium ascorbate solution, and the reading at the very surface was used to represent 100% O₂ saturation. Fine scale readings were made in the laboratory and in the field using a micromanipulator (Unisense, Denmark) or by simply inserting the electrode to the desired depth by hand. Depths were expressed relative to the soil surface. O₂ profiles were measured at several locations along three separate transects across the southern, central, and northern parts of the DTLB. Similarly, a small set of H₂ measurements were made using a Unisense microelectrode and picoammeter. Because of the very low H₂ concentrations measured (lower than ambient atmospheric concentrations), water in equilibrium with the atmosphere was used as a standard, assuming a value of 0.55 ppm for the atmosphere and solubility based on the ambient temperature. Oxidation-reduction potential (ORP) measurements were made with a Thermo-Orion (Waltham, MA) electrode and portable multimeter. Measurements were taken at 2, 5, and 10 cm depths at various locations across the three transects.

2.3. Soil Iron Fractionation and Analysis

[8] Three replicate soil cores were taken to depth of 50 cm using a SIPRE corer during the winter and shipped frozen by air courier to the laboratory, where they were further divided into five 10 cm depth increments. Each horizon was further subdivided longitudinally (while still frozen), and subsamples were subjected to a variety of extractions to determine the presence of Fe minerals along the soil profile. One subsample was extracted in 1 N sulfuric acid for 24 h, and total Fe was analyzed by the phenanthroline method described above. Other subsamples were freeze-dried in a 12 L capacity Freezone freeze-drier at -50°C, <70 micro-bars pressure. Each of the depth increments was lightly sieved to <2mm in a brass sieve, with the main materials removed being large tangles of roots in the upper 15 cm of the profiles. Replicate sieved samples of 0.250–0.300 g were extracted with either (1) 0.2 M ammonium oxalate/0.17 M oxalic acid (pH 3.2) in a darkened cabinet at RT, (2) sodium acetate/HAc (pH 4.50) for 48h at 50°C in a reciprocating shaker, or (3) sodium dithionite/sodium citrate overnight at RT [Poulton and Canfield, 2005]. The soil suspensions were vortexed and centrifuged at 3500 × g for 6 min, and the supernatant was decanted. Extracts were diluted 2-fold after correcting for solution entrainment gravimetrically and filtered to 0.22 microns using nylon syringe filters. The filtrates were stored at 4°C briefly until measurement. Filtrates were analyzed for Fe (259.9 nm), Al (396.1 nm), and S (180.7 nm) in a Thermo Jarell Ash IRIS Advantage inductively coupled plasma emission spectrometer (ICP-OES) whose optics had been purged with dry Ar. S was not determined on the dithionite/citrate extracts. Samples and extraction blanks were interspersed with authentic Fe/Al/S standards derived from NBS-traceable stocks (10,000 ppm; VWR). Extraction blanks were low and, in the case of Fe, nondetectable. S was only rarely detected in significant amounts and then only in the Na-acetate extracts of peats. Fe contained in these fractions was converted to a volumetric basis based on bulk density measurements.

[9] Samples of the organic horizon (15 cm depth) were collected on three dates in 2009 (27 June, 14 July, 3 August)

from 16 to 18 locations across the landscape using a serrated knife. Soils were sealed in Bitran specimen bags, frozen, and shipped to the laboratory where Fe was extracted with 1 N sulfuric acid as described above.

2.4. Description of Soil Bacterial Community

[10] We collected samples of the organic layer from various locations at each of three transects across the DTLB on two dates in 2005 (13 June and 29 August). DNA was extracted from each sample individually using an alkaline lysis technique, gel purified using glass beads, then 16S rRNA genes were amplified by polymerase chain reaction (PCR) using universal eubacterial primers F27 and R1510 [Lipson *et al.*, 2008]. PCR products of spatial replicates were then combined, gel purified, and cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad CA), producing separate libraries for the two dates. Inserts were partially sequenced, compared with the public database using BLAST (www.ncbi.nlm.nih.gov/BLAST), aligned using Nstaligner (greengenes.lbl.gov) [DeSantis *et al.*, 2006], and placed into neighbor-joining and maximum parsimony phylogenetic trees. The June and August communities were compared with the PTP test [Martin, 2002], using the PAUP software package (Sinauer Associates, Sunderland, MA). The sequences were submitted to GenBank and given the accession numbers HQ162140–HQ162262.

2.5. Laboratory Microcosm Experiments

[11] A series of experiments were performed in laboratory incubations on previously frozen soils. These experiments were designed to measure the net rates of Fe reduction in soils and relate these to rates of soil respiration. For all these experiments, soil cores were collected in the field from areas of high or low topography along three transects, sealed in polyethylene bags, and frozen. In two experiments, net rates of Fe reduction were measured. In the first of these experiments, frozen cores were shaved to fit into 4 cm inner diameter, ~12 cm long polyvinyl chloride (PVC) tubes, which were capped and sealed at the bottom, and allowed to thaw in a 4°C incubator. Upon thawing, a soil moisture sampler was inserted vertically into the center of each core. Cores were brought up to full saturation by adding sterile distilled water and were allowed to equilibrate for several days at 10°C under a normal atmosphere. Cores were injected with 5 cm³ of 0.1 M ferric citrate, using a 5 cm long hypodermic needle, yielding a theoretical final soil pore water concentration of approximately 5 mM Fe(III). Soil pore water samples (~1 mL volume) were collected repeatedly over the following 5 days after injection into small, sterile evacuated tubes. A drop of 0.1 M HCl was added as preservative, and Fe(II) concentrations were measured by 1,10-*o*-phenanthroline as described above. CO₂ flux was measured by attaching a cuvette, fashioned from PVC to fit tightly over the cores, and monitoring changes in CO₂ using a portable IRGA (EGM-1, PP systems, Amesbury, MA). In a second set of Fe reduction experiments, frozen soils were placed into mason jars, whose lids were fitted with septa, and the headspace was flushed with N₂ gas for 2 min to create anaerobic conditions (verified with indicator strips placed inside each jar). Soils were allowed to thaw at 4°C overnight. After thawing, ferric pyrophosphate (Fe₄(P₂O₇)₃) was added to soils to ~5 mM final Fe(III)

concentration by injecting with a syringe through a rubber septa on the lid, flushed again with N_2 to remove gases that accumulated during thawing, and incubated at 10°C . The switch to ferric pyrophosphate for the second set of experiments was made based on the observation that ferric citrate was less effective in culturing Fe(III)-reducing bacteria (Jha, unpublished data), probably owing to the fact that citrate binds somewhat strongly to Fe(III), possibly reducing its availability [Neilands, 1981]. The counterion, pyrophosphate, could have an impact on soil processes, though a recent study from the same area showed no short-term effects of mineral nutrient (NPK) addition on soil respiration or bacterial biomass [Allen *et al.*, 2009]. Gas samples were collected with syringes after 6 and 24 h, and after the 24 h sample, soil water was collected, Fe(II) was analyzed by 1,10-*o*-phenanthroline. Initial Fe(II) concentrations from the time of soil collection were subtracted to calculate net Fe reduction rates. CO_2 and CH_4 were analyzed by gas chromatography (Model 8610C with methanizer and flame ionization detector, SRI Instruments, Torrance, CA, using recommended protocols of manufacturer).

[12] A separate pair of experiments was performed to test the effect of various e^- acceptors on substrate-induced respiration (SIR). In these experiments, soils were thawed and weighed into sidearm flasks, and a mixture of ^{14}C -labeled and unlabeled glucose solution was added along with mineral nutrients (10 mM NH_4Cl , 1 mM KH_2PO_4) and the e^- acceptor or control solution. Soils were incubated at 10°C . The sidearm, containing 1 M NaOH to trap evolved CO_2 , was sampled and replaced at regular intervals, and $^{14}\text{CO}_2$ was analyzed by liquid scintillation counting. In the first experiment, 2 mg glucose C g^{-1} soil was added, an amount previously shown to maximize initial SIR in these soils. The treatments consisted of either aerobic incubation with water added in place of electron acceptor or incubation under N_2 atmosphere with water alone, ferric pyrophosphate, or Mn (IV) oxide (MnO_2 , 60–230 mesh, Sigma-Aldrich, St. Louis, MO) added as powder (8.7 mg g^{-1} soil) with an equivalent volume of water. In the second SIR experiment, the amount of glucose was increased to 4 mg C g^{-1} soil, after observing that respiration rates declined quickly in the first experiment. This experiment was designed to test whether insoluble Fe(III) oxides or the soluble redox shuttle, anthraquinone-2,6-disulfonate (AQDS), frequently used as an analog humic e^- acceptor [Lovley *et al.*, 1996], would stimulate SIR. The treatments included AQDS (final concentration, 500 μM), Fe_2O_3 (hematite, $<5 \mu\text{m}$, Sigma-Aldrich, St. Louis, MO) added in powder form (10.4 mg g^{-1} soil) with an equivalent volume of water, both hematite plus AQDS, and a control with only water. In this experiment, all incubations were carried out under an N_2 atmosphere.

2.6. Field-Based Fe(III)-Reduction Experiments

[13] Two field experiments were performed to measure transformations of Fe in situ, and the effects of various amendments on soil respiration. The first experiment was performed on 18 July 2008. PVC collars (10 cm diameter, 12 cm long) were carefully inserted into the soil using a serrated knife, and a soil moisture sampler was inserted vertically into the center of each. Two plots were established, both within low centered polygons near the DTLB

margin. One plot contained six replicate cores separated by >1 m and was used to measure net rates of Fe reduction after injection of ferric pyrophosphate (25 mM, 4×10 mL per core injected into cardinal points using a syringe with 10 cm long stainless steel tube). Soil water samples (~ 1 mL) were collected into small vacutainers immediately before injection and after 3, 24, 48, and 69 h. A drop of 0.1 M HCl was injected by syringe into each vacutainer immediately after sampling, and samples were kept cold until analysis 1 week later. Soil respiration was measured before injection and at the time of each soil pore water collection using a portable IRGA (EGM-4 with SRC-1 soil chamber, PP Systems), using a quadratic fit to estimate initial respiration rates. ORP was measured next to each replicate before injection and in the center of each plot, as well as in uninjected areas within the same polygon, at the end of the experiment. The second plot contained five clusters with three collars in each. Each cluster was separated by at least 5 m, and adjacent collars within a group were separated by at least 1 m. The three cores in each cluster received 80 mL of sterile water, glucose (70 mM), or glucose + mineral nutrients (70 mM glucose, 186 mM NH_4Cl , 5.3 mM KH_2PO_4). Soil respiration was measured immediately before injection and after 4, 27, and 78 h.

[14] A second field Fe-reduction experiment was performed on 25 July 2009. This experiment was similar to the first, except the treatments consisted of four replicates each of either water, $\text{Na}_4\text{P}_2\text{O}_7$ (final concentration, 3 mM), $\text{Fe}_4(\text{P}_2\text{O}_7)_3$ (final concentration, 3 mM), and EDDS (a biodegradable alternative to EDTA used as a chelator of Fe, 1 mM final). Additionally, the PVC collars used in this experiment extended to 15 cm in depth. Soil water and respiration rates were sampled immediately before injection of the treatment solutions and again after approximately 3 and 24 h.

2.7. Statistical Analysis

[15] Seasonal and spatial patterns of soil pore water Fe were tested using two-way analyses of variance (ANOVA), with date and topographic type (high versus low) as categorical variables. Post-hoc pairwise comparisons between specific dates were made using Fisher's least significant difference test. Regressions among variables were performed using the General Linear Model procedure of Systat. The SIR experiments were analyzed using analysis of covariance (ANCOVA), with time as the covariate and treatment as a categorical variable. Where necessary, data were log transformed prior to analysis. Other statistical details are given where relevant.

3. Results

3.1. Soil Redox Conditions and e^- Acceptor Availability

[16] We found that anaerobic conditions predominated across the DTLB and that Fe reduction is likely a major respiratory pathway given soil redox conditions and concentrations of e^- acceptors in the soil water. Within DTLBs, except in raised areas such as polygon rims, the soil is generally saturated in midsummer [Hinkel *et al.*, 2001; Zona *et al.*, 2009], and accordingly, O_2 generally dropped to very low levels within 1–4 cm depth (Figure 1). Similarly, at

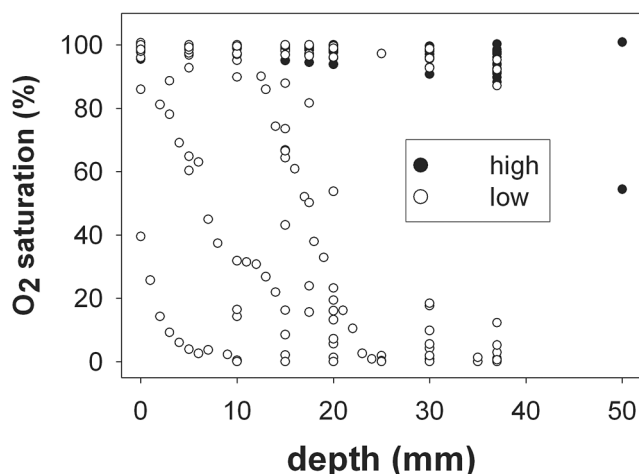


Figure 1. Oxygen profiles in areas of high or low micro-topographic features, measured in July 2007. The soil surface = 0 cm.

most depths and locations across the landscape, ORP was generally too negative for aerobic respiration and fell within a range that would favor Fe(III) reduction [Kappler *et al.*, 2004; Zehnder and Stumm, 1988] (Figure 2). Dissolved H_2 was measured on two dates: on 2 August 2007, the mean concentration at 3.7 cm depth (of four locations) was 0.30 ± 0.08 nM, and on 14 July 2008, the mean at 5–15 cm depth (of 10 locations) was 0.29 ± 0.01 nM (data not shown). H_2 concentrations in this range are also consistent with Fe(III) reduction [Lovley *et al.*, 1994]. Among potential e^- acceptors, Fe(III) dominated the soil pore water (Table 1), with surprisingly high concentrations given the moderately acidic pH of the soil. To verify the results obtained using the colorimetric phenanthroline method, total Fe was measured on a subset of samples using ICP, and both methods produced comparable results (ICP, 330.8 ± 45.4 μ M; phenanthroline, 350.7 ± 65.1 μ M). In the majority of soil pore water samples, dissolved S concentrations (measured by ICP) were below detection limits, with a small number of outliers having higher values. Sulfate was below detection limits using a turbidometric method (the lowest standard used was 10 μ M, data not shown). Similarly, most samples had NO_3^- concentrations near detection limits, but two outliers increased the mean.

[17] Mean DOC levels in soil pore water were not significantly different between high and low areas; however, water from low areas (e.g., low centered polygons) had significantly higher UV absorbance ($P = 0.003$, Table 1). DOC and A_{260} were significantly correlated ($R^2 = 0.481$, $P < 0.001$; $R^2 = 0.644$ when several outliers removed; data not shown). A_{260} was significantly related to soil pore water concentrations of Fe(III), though the relationship varied by date (Figure 3). A statistical model that included date, Fe(III) and their interaction explained 95.9% of the variation in A_{260} , with the Fe(III) and interaction terms highly significant. The equivalent model for Fe(II) was not significant.

[18] The pore water concentrations of Fe(II), Fe(III), and total Fe varied considerably over the course of this study and showed a trend toward being most oxidized later in the summer (Figure 4). This shows that Fe cycles dynamically

between oxidized and reduced forms in this system. In two-way ANOVA testing, the effects of date and topography, Fe(II), Fe(III), and the ratio of Fe(II)/total Fe varied significantly between dates ($P < 0.001$). The ratios of Fe(II)/total Fe on the final dates of 2006 and 2007 were significantly lower than on previous dates. Fe(III) was significantly lower in areas of high topography ($P = 0.007$), while the difference for Fe(II) was only marginally significant ($P = 0.058$), and Fe(II)/total Fe was not significantly affected by topography.

3.2. Fe Minerals in Soil Profile

[19] Fe-bearing minerals were found in all layers of the soil profile (Figure 5). While the mineral layers (below 20 cm) had the most, the organic layer contained significant amounts as well. The dithionite-citrate extraction, which yields highly reactive Fe oxyhydroxides, including the “easily reducible” ferrihydrite and lepidocrocite and the “reducible” goethite and hematite [Poulton and Canfield, 2005], recovered measurable quantities of Fe in all layers. The acetate treatment extracts mainly the Fe(II)-containing mineral, siderite ($FeCO_3$), which also appeared in all layers. The oxalate extraction recovered much more Fe than the dithionite-citrate extraction in the deepest layers, possibly indicating the presence of magnetite in the permafrost (>30 cm depth), while both of these fractions may contain reducible Fe oxides, magnetite is extracted only by oxalate [Poulton and Canfield, 2005]. The slightly higher levels of acid-extractable compared to oxalate-extractable Fe at the deepest layer could possibly indicate the presence of sheet silicate minerals, like biotite. Al was present in much higher concentrations in dithionite-citrate extracts of the mineral layers relative to the upper layers (data not shown), indicating the presence of aluminosilicate minerals. S was present mainly in acetate extracts, at low concentrations (71 times less than Fe).

3.3. Seasonal Changes in Extractable Fe

[20] There was a pattern of Fe reduction in acid-extractable Fe in the organic layer over the summer (Figure 6). Total Fe levels stayed fairly constant from late June to early

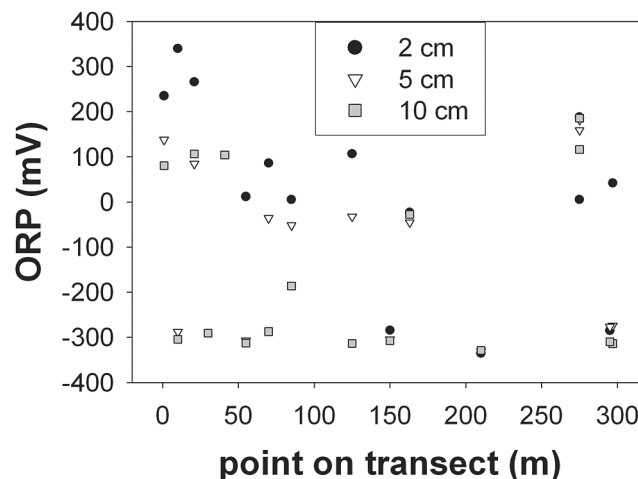


Figure 2. Oxidation-reduction potential (ORP) at three depths, across three transects. Measured in July 2006.

Table 1. Soil Pore Water Concentrations (Means and Standard Errors) of Elements or Chemical Species That May Serve as e⁻ Acceptors (μM), Dissolved Organic C (mg/L), and UV Absorbance (A_{260} , Expressed in μM Equivalents of Tyrosine) From Areas of High or Low Microtopography^a

Topography	Fe(III) (μM)	Mn (μM)	S (μM)	NO_3^- (μM)	NO_2^- (μM)	DOC (mg/L)	A_{260} (μM Tyr)
High	168 \pm 44	2.1 \pm 0.7	42.0 \pm 31.1	6.0 \pm 1.9	0.62 \pm 0.11	40.5 \pm 7.2	522 \pm 89
Low	343 \pm 54	2.2 \pm 0.8	10.6 \pm 6.6	12.7 \pm 7.4	0.76 \pm 0.28	42.3 \pm 4.7	977 \pm 120
Median	140	2.3	0.0	5.4	0.54	36.4	503.9
<i>n</i> (H, L)	(28, 35)	(8, 8)	(8, 8)	(10, 11)	(9, 9)	(26, 30)	(30, 34)

^aThe median of all measurements is given to indicate the effect of a small number of outliers. The sample size (*n*) is given for the two topographic categories for each variable.

August, but Fe(III) declined while Fe(II) increased. On the basis of either the Fe(II) increase of 54 $\mu\text{mol g}^{-1}$ or the Fe(III) decrease of 80 $\mu\text{mol g}^{-1}$ over this 36 day period, this implies an average Fe reduction rate of 0.2–0.3 $\mu\text{mol g}^{-1} \text{s}^{-1}$ in the organic layer.

3.4. Soil Bacterial Community

[21] We described the bacterial community using molecular analyses (16S rRNA genes) of soil combined from numerous samples collected across the landscape, on two separate dates in 2005 (Figure 7). This analysis revealed the presence of various bacteria likely involved in both reduction and oxidation of Fe. The most abundant ribotype in the late summer (6 of 65 sequences) was closely related (>97% similarity) to *Rhodoferrax ferrireducens*, a bacterium capable of anaerobically reducing Fe(III) [Finneran *et al.*, 2003]. There were also other several sequences whose closest BLAST match was to a likely transformer of Fe. Two June sequences, from the Acidobacterial and Gammaproteobacterial lineages, were most closely related (>97%, >92.5% similarity, respectively) to uncultured sequences discovered in an Fe redox front in a sedimentary mineral matrix, known to contain both Fe reducing and oxidizing microbes [Yoshida *et al.*, 2006]. A cluster of sequences, containing four from June and two from August, had as their closest relative in the database (>95% similarity) a sequence from a runoff stream at Iron Mountain, whose community is primarily made up of Fe oxidizing bacteria [Edwards *et al.*, 1999]. Also potentially of note, two August sequences had as their closest cultured relative the Fe oxidizer, *Ferrimicrobium*. However, this relationship was more distant than those previously mentioned (~80% similarity). On the basis of the PTP test, the communities differed significantly between June and August ($P = 0.036$).

3.5. Laboratory-Based Measurements of Fe Transformations

[22] After having established that soil chemistry was favorable for Fe reduction and that Fe-transforming bacteria appeared to present in the community, we performed a series of experiments to measure the rates of Fe reduction in soil. The first set involved soil cores in PVC cores that were water saturated but incubated aerobically. In the second set soils were incubated under a N_2 atmosphere. The results are summarized in Table 2. In the incubations in which the surface of the soil cores were exposed to the atmosphere, net Fe(III) reduction occurred initially in these incubations (0–72 h), but reoxidation was observed thereafter (data not shown). Measurements of the O_2 profile in these cores

showed similar patterns to those observed in the field: O_2 dropped to low levels within 3 cm of the surface. Therefore, the majority of the core was still essentially anaerobic. Net Fe reduction and respiration rates in the experiments with fully anaerobic incubations were not significantly different from the first set of experiments, and so the two sets are combined in Table 2. Conservative estimates of the fraction of respiration that could be accounted for by the net Fe reduction rates averaged 4.5% but were as high as 23.3% in some samples. However, these values are probably underestimates, as both oxidation and reduction appeared to be occurring simultaneously, even in largely anaerobic soils (among other reasons, see discussion). In the experiment in which net Fe reduction and methane flux were measured in the same samples, the values were 0.149 ± 0.019 and $0.049 \pm 0.005 \mu\text{mol g}^{-1} \text{h}^{-1}$, respectively. Assuming that two thirds of methanogenesis occurs by the acetoclastic pathway and one third by the hydrogenotrophic pathway, [Conrad, 1999; Metje and Frenzel, 2007], each mole of CH_4 represents about 3.33 mol e⁻, and so net rates of e⁻ flow to Fe(III) and to CH_4 were roughly equivalent (0.149 ± 0.019 and $0.163 \pm 0.017 \mu\text{mol g}^{-1} \text{h}^{-1}$, respectively).

[23] In further experiments to explore the effect of e⁻ acceptor availability on respiration, we performed SIR experiments in which soils were incubated with ^{14}C -glucose in the presence of various e⁻ acceptors. In the first of these experiments, the soils incubated with the soluble Fe(III) salt, $\text{Fe}_4(\text{P}_2\text{O}_7)_3$, under a N_2 atmosphere produced the most $^{14}\text{CO}_2$ over the course of the experiment, surprisingly, more

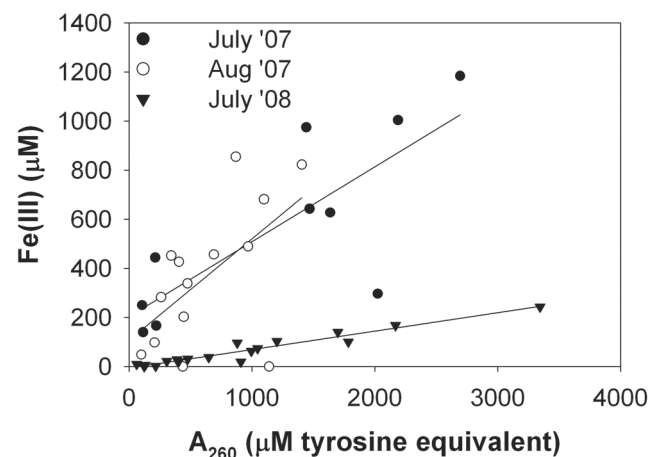


Figure 3. Relationship between Fe(III) concentration and UV absorbance (A_{260}) in soil pore water on three dates.

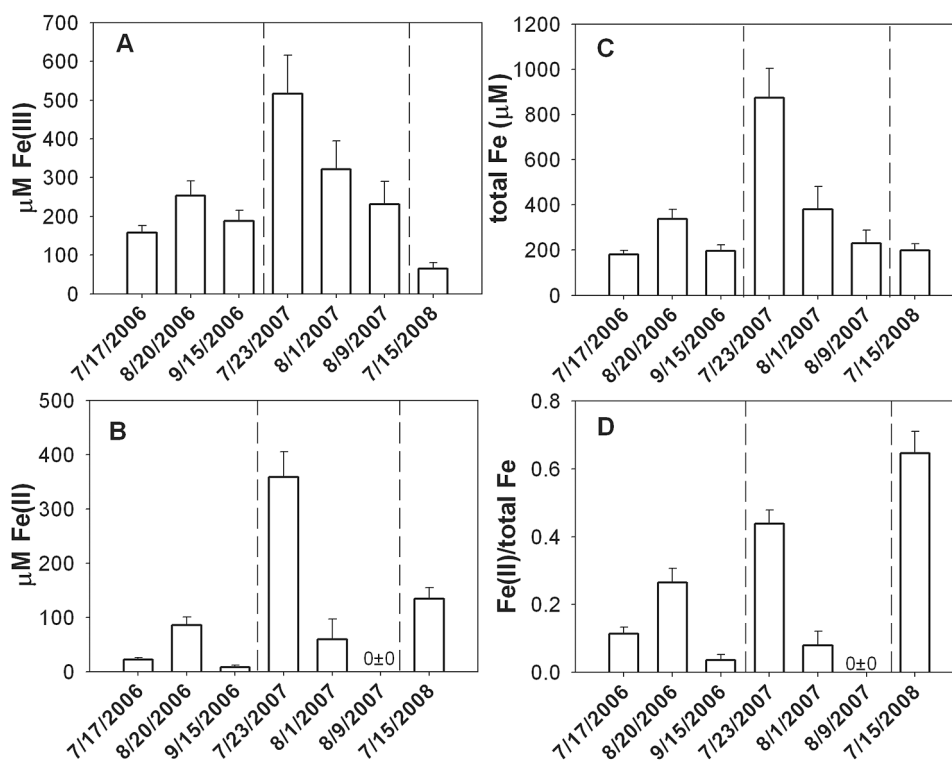


Figure 4. Concentrations of soluble Fe species in soil pore water on various dates from 2006 to 2008. (a) Fe(III); (b) Fe(II); (c) total Fe, Fe(II) + Fe(III); (d) ratio of Fe(II) to total Fe. Values are means with standard errors.

than soils incubated aerobically (Figure 8a). The accumulation of $^{14}\text{CO}_2$ over time fit a logarithmic curve, and so when these data were replotted by log-transforming the time axis, the result was highly linear ($R^2 = 0.978$). This allowed us to perform an ANCOVA that confirmed that the treatment \times time interaction was significant ($P < 0.001$), meaning the e^- acceptors did indeed impact the rate of respiration. In the second SIR experiment, more glucose was added so that a more sustained period of maximal respiration could be

observed. In this experiment the purpose was to determine whether soil microbes could use insoluble Fe(III) hydroxides (hematite), AQDS (a soluble humic analog known to be used as an e^- shuttle by Fe and humic-reducing bacteria) [Lovley *et al.*, 1996], or both in combination. In Figure 8b, the Y axis shows the rate of $^{14}\text{CO}_2$ evolution (rather than accumulation over time as in Figure 8a), which increased linearly over time in the two treatments that contained AQDS and decreased over time in the treatments without

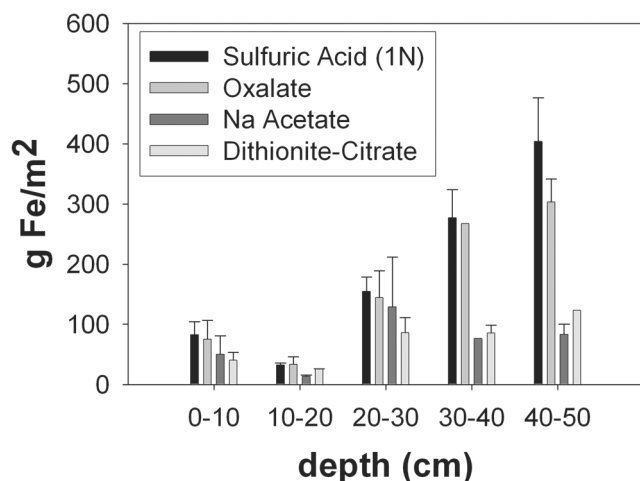


Figure 5. Fe extracted using a variety of fractionation techniques along a soil profile. See text for interpretation.

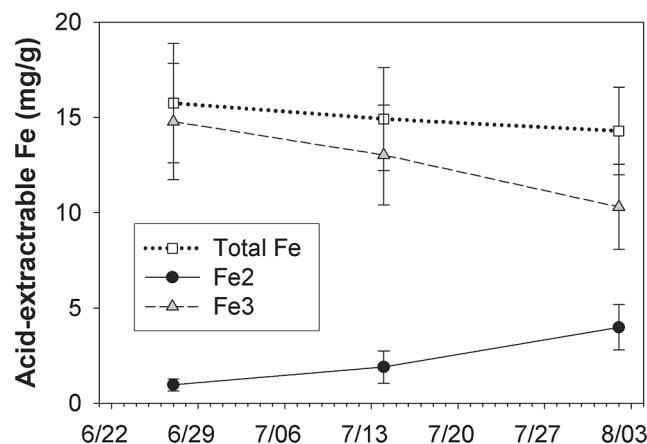


Figure 6. Seasonal changes in acid-extractable Fe from organic layer (upper 15 cm) of soil in 2009. ($N = 16$ –18 per date).

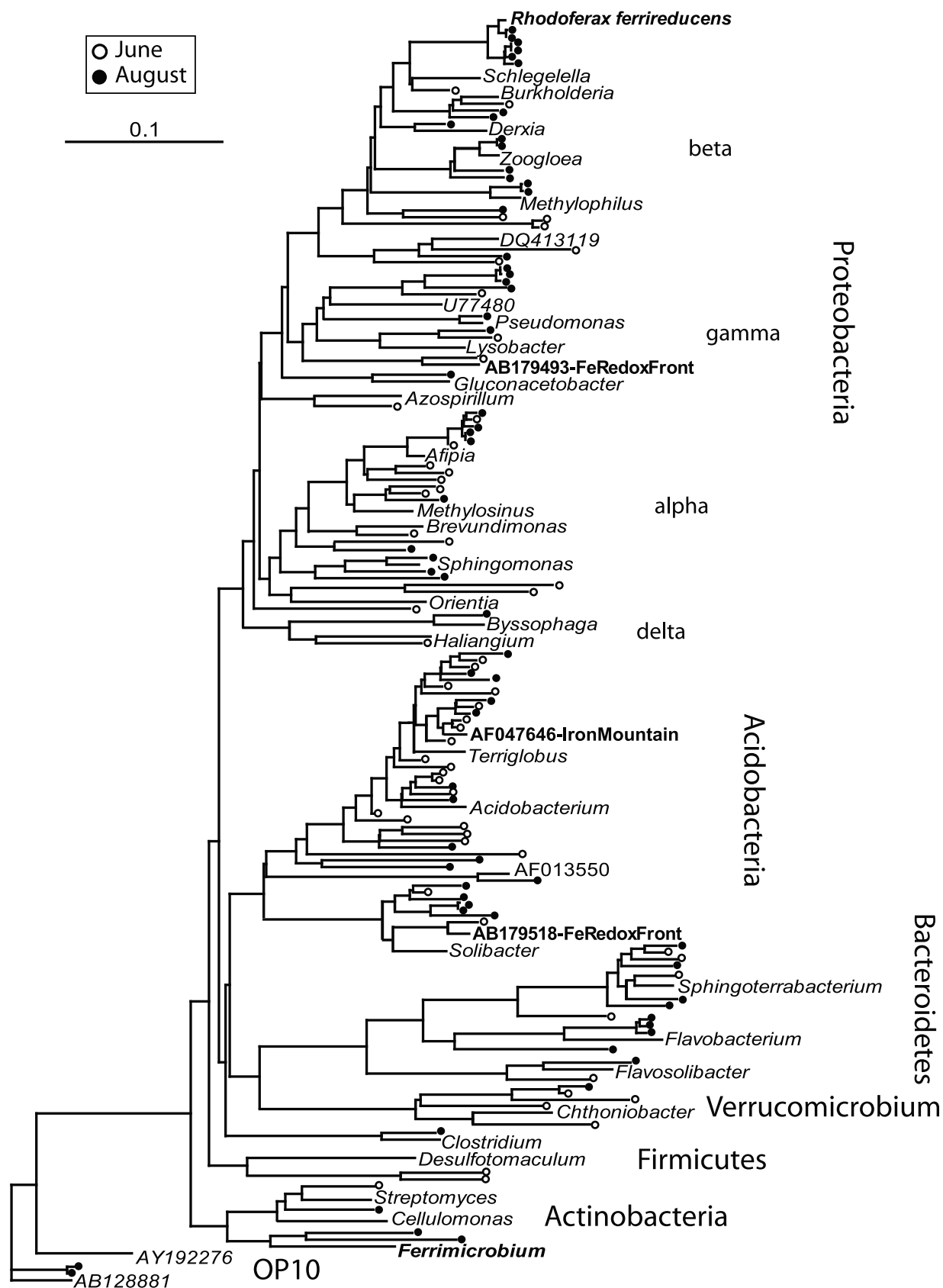


Figure 7. Neighbor-joining phylogenetic tree of bacterial 16S rRNA sequences from soils collected during June and August 2005.

Table 2. Summary of Laboratory Fe(III) Reduction Experiments, Showing Net Rate of Fe(III) Reduction ($\mu\text{mol Fe g}^{-1} \text{ h}^{-1}$), Respiration Rate ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ h}^{-1}$), and Fraction of Respiration Accounted for by e^- From the Net Fe(III) Reduction Rate

	Fe(III) Reduction	Respiration	e^- Fraction
Mean	0.14	1.22	0.045
SE	0.02	0.15	0.010
Min	0.04	0.06	0.004
Max	0.37	4.11	0.233

AQDS. The treatment \times time interaction was significant ($P < 0.001$). Apparently, the microbial community was able to use the soluble quinone, AQDS, as e^- acceptor, but not the form of insoluble Fe oxide added in this experiment.

3.6. Field-Based Experiments of Fe Transformations

[24] The soils used in the above laboratory-based experiments were, by necessity, disturbed, and so we performed two experiments in situ in the field to measure rates of Fe transformation and link them to soil respiration. In the first experiment we followed changes in Fe(II) and Fe(III) concentrations in soil pore water and in soil respiration after injecting $\text{Fe}_4(\text{P}_2\text{O}_7)_3$. Of the six replicates, two actually showed small initial decreases in Fe(II), though on average, there was an initial period of net reduction (Figure 9a). After the initial reduction phase, the ratio of Fe(II)/total Fe gradually declined from 0.36 to 0.18, despite the fact that the overall ORP of the injected areas became more negative. The ORP of the study area was initially -58.8 ± 10.7 mV, which dropped to -125.9 ± 12.0 mV in areas that had been injected, while uninjected areas remained essentially unchanged, at -55.1 ± 10.4 mV. The fact that Fe became more oxidized over time (after the initial reduction phase) while the overall ORP was becoming more reduced implies that soluble Fe may serve as an intermediate e^- carrier, donating e^- to some component of the soil, such as quinone moieties of humic substances [Peretyazhko and Sposito, 2006]. Upon injecting $\text{Fe}_4(\text{P}_2\text{O}_7)_3$ into the soil, respiration increased 33.3% (Figure 9b) and remained at that level for the duration of the experiment (3 days). During the initial phase, net Fe reduction proceeded at a rate of $2.46 \pm 1.77 \mu\text{mol m}^{-2} \text{ s}^{-1}$, theoretically accounting for an average of $83.8 \pm 53.7\%$ of soil respiration (the high variability was due to the two of six replicates where Fe(II) initially declined). After injection, soil respiration was related to soil pore water concentrations of Fe(III) ($R^2 = 0.36$, $P = 0.002$) at the time of each measurement, providing more evidence that increased Fe availability stimulated respiration in these soils. In a separate experiment that took place during the same time a short distance away, injections of water alone, glucose solution, or a combined glucose/nutrient solution failed to produce increases in respiration, and respiration rates in soils injected with Fe(III) were higher than these other treatments. (The treatment effect was significant at $P < 0.001$ in an ANCOVA with time as the covariate. According to pairwise Fisher's least significant difference tests, respiration in the Fe(III) treatment was greater than all others, and respiration in the glucose treatment was higher than the water treatment). These results indicate that

respiration was more limited by availability of e^- acceptors than by energy or mineral nutrients.

[25] A second field-based Fe reduction experiment conducted in 2009 gave similar results (Table 3). In this experiment, only one replicate (in the $\text{Na}_4\text{P}_2\text{O}_7$ treatment) showed a slight decline in Fe(II) over the initial 3.17 h period, while net Fe reduction occurred in all other replicates. Adding chelated Fe(III) (ferric pyrophosphate) increased net Fe reduction rates relative to the water control. Chelators alone (sodium pyrophosphate and EDDS) also stimulated Fe reduction, though to a lesser extent. Respiration appeared to be stimulated by addition of EDDS and chelated Fe(III); this effect was marginally significant ($P = 0.069$). The Fe reduction rates calculated from the initial 3.17 h interval could account for all of the measured soil

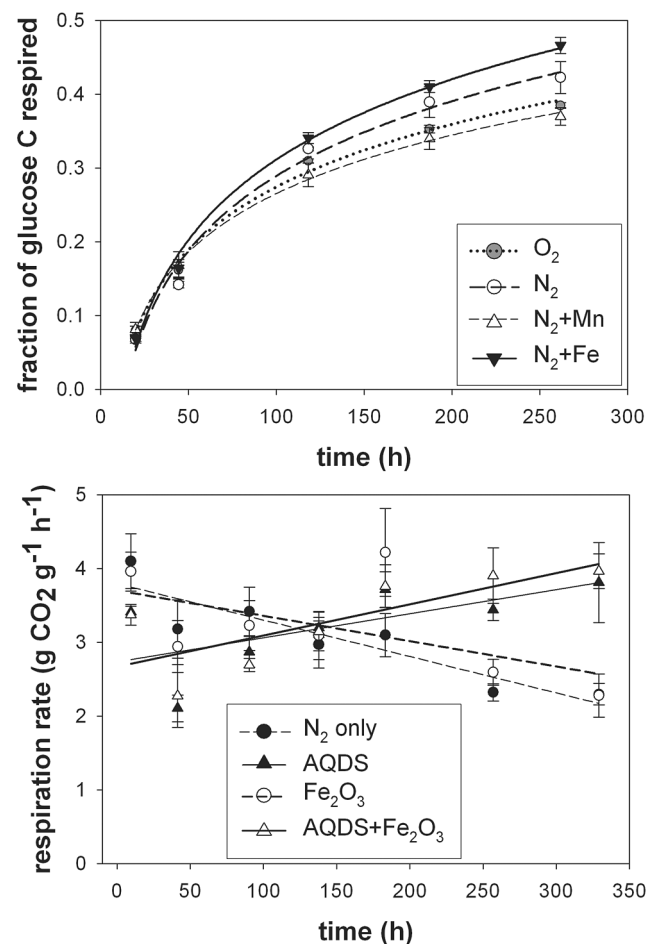


Figure 8. Results of two substrate-induced respiration experiments in which availability of e^- acceptor was varied, and respiration of ^{14}C -glucose was monitored. (a) SIR experiment 1, in which soils were either incubated aerobically (O_2), or under N_2 atmosphere, with ferric citrate ($\text{N}_2 + \text{Fe}$), Mn oxide ($\text{N}_2 + \text{Mn}$), or a nutrient solution with no additional e^- acceptors (N_2). Two mg glucose C g^{-1} soil was added, and data are expressed as cumulative fraction of recovered ^{14}C . (b) SIR experiment 2, in which soils were incubated under N_2 atmosphere and amended with insoluble Fe_2O_3 , AQDS, or both. Four mg glucose C g^{-1} soil was added, and data are expressed as rates of $^{14}\text{CO}_2$ evolution.

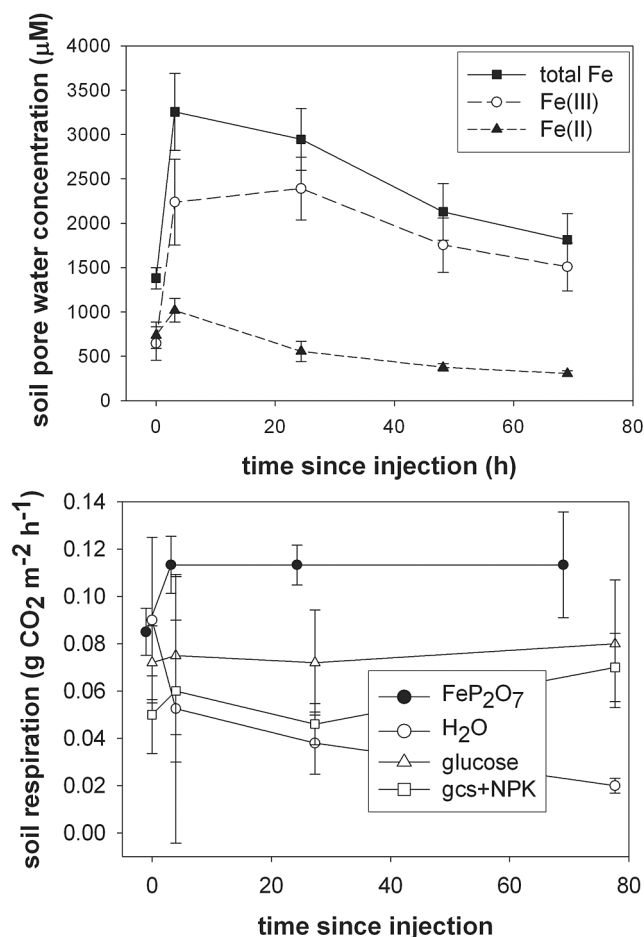


Figure 9. Field-based Fe(III) reduction experiment performed on 18 July 2008. (a) Soil pore water Fe pools before and after injection with $\text{Fe}_4(\text{P}_2\text{O}_7)_3$. (b) Respiration in soils before and after injection with H_2O , $\text{Fe}_4(\text{P}_2\text{O}_7)_3$, glucose, or glucose + mineral nutrients.

respiration in the Fe(III) treatment, while the net Fe reduction rates observed in the water control accounted for about 13.5% of soil respiration.

4. Discussion

[26] On the basis of redox conditions, availability of e^- -acceptors in the soil pore water and solid phase, composition of the microbial community and activities measured in

laboratory and field experiments, Fe reduction is an important pathway for respiration in this Arctic peat soil. This has major implications for understanding the controls over CO_2 and CH_4 flux from this ecosystem. For one, anaerobic respiration is potentially a major pathway for CO_2 loss from this ecosystem type. The in situ Fe reduction experiments showed that, given an adequate supply of Fe (III), a large portion of soil respiration can be driven by Fe reduction. The reducible Fe oxides we observed in the soil profile could allow for significant anaerobic respiration during flooded periods. On the basis of the dithionite-citrate fraction (which is specific for reducible Fe hydroxides) in the active layer (0–30 cm), 151 g Fe/m^2 is available for reduction. Assuming this pool cycles annually with the rise and fall of the water table, a 100 day summer and C with an oxidation state of zero, this would account for about 0.08 g $\text{CO}_2 \text{ m}^{-2} \text{ d}^{-1}$, or about 15% of typical ecosystem respiration rates (Oechel, unpublished data), or about 30% of heterotrophic respiration, assuming it is 50% of ecosystem respiration [Hanson *et al.*, 2000]. The seasonal changes in reduction state of acid-extractable Fe in the organic layer show that such transformations do indeed occur. In fact, the aforementioned estimate may be conservative, as the observed reduction of solid phase Fe from the upper 15 cm alone could represent 20%–30% of heterotrophic respiration during July. Furthermore, in short-term anaerobic incubations, microbes sustained rates of respiration that were higher than rates in aerobic treatments. This indicates that much of the microbial community is obligately anaerobic and is therefore inhibited by oxygen. Respiration in these soils may be less limited by e^- acceptor availability than generally assumed, and this could have implications for responses to climate-induced changes in water table depth in this ecosystem.

[27] The importance of Fe in respiration also has implications for the impact of a warmer Arctic: a deeper thaw may provide more contact with the mineral layer, allowing a higher availability of Fe. The origin of Fe in the organic layer in this system is not completely certain. Dust inputs and sediment from the Brooks Range are possible sources, but the mineral layer underlying the organic horizon is the most obvious proximate source, as it contained significant amounts of Fe hydroxides and other Fe-containing minerals. The DTLB studied here was of medium age, and so the active layer included mineral material. The effects of climate change could be most drastic in older basins with thicker organic layers [Hinkel *et al.*, 2003], where a small increase in thaw depth could lead to a large increase in Fe availability by thawing previously unavailable mineral layers.

Table 3. Results of Fe Reduction Experiment Performed on 25 July 2009, Showing Rates of Fe Reduction and Soil Respiration Over 4 h After Injection With Solutions Shown at Left^a

Treatment	Fe Reduction Rate ($\mu\text{mol Fe m}^{-2} \text{ s}^{-1}$)	Soil Respiration Rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	e^- Fraction (%)
Water	0.319 (0.138)	0.669 (0.182)	13.5 (5.8)
$\text{Na}_4\text{P}_2\text{O}_7$	1.731 (0.667)	1.196 (0.506)	37.1 (18.1)
EDDS	3.228 (0.645)	1.103 (0.214)	74.9 (15.7)
$\text{Fe}_4(\text{P}_2\text{O}_7)_3$	5.323 (0.762)	1.313 (0.242)	125.8 (23.7)
<i>F</i>	13.153	3.073	8.493
<i>P</i>	<0.001	0.069	0.003

^aThe last column shows the percentage of soil respiration that could be accounted for by Fe reduction. Values are means and standard errors of four replicates. *F* and *P* statistics are given for the treatment effects in one-way ANOVAs (*df* = 3, 12).

[28] Finally, the major role of Fe(III) and humic materials as e⁻ acceptors in Arctic peat has important implications for CH₄ flux. Methane production can be considered a thermodynamic “last resort,” which is only favorable when no other e⁻ acceptors are available. Fe and humic reduction competes with methanogenesis for substrates such as acetate or molecular hydrogen [Bond and Lovley, 2002; Cervantes et al., 2000; Teh et al., 2008], and so regulation of Fe availability is likely to be an important control over CH₄ production in this ecosystem. On the basis of our conservative measurements of net Fe reduction rates, this process is comparable in magnitude to methanogenesis.

[29] Directly estimating Fe reduction rates in this system was challenging, particularly in laboratory-based experiments. While Fe reduction could explain much of observed soil respiration in field experiments, a much lower fraction was found in laboratory studies. Fe reduction rates measured in laboratory incubations (converting from Table 2, 0.35 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were comparable with rates observed in the water controls of the 2009 field experiment (Table 3, 0.319 $\mu\text{mol m}^{-2} \text{s}^{-1}$). However, respiration rates measured in the laboratory were much higher (3.0 versus 0.67 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Clearly the disturbance associated with collecting, freezing, storing, and thawing soils can stimulate respiration rates measured in the laboratory. There are additional reasons our estimates of the contribution of Fe reduction to CO₂ flux may be conservative: (1) Fe oxidation and reduction appear to occur simultaneously, even in largely anaerobic soils. As discussed below, reduced Fe could possibly be reoxidized by some organic soil component; (2) based on the high amounts of siderite observed in the soil profile, some reduced Fe may be retained in the solid phase and therefore not detected in our assays of soil pore water; (3) the organic substrate for the process was assumed to have C with an average oxidation state of 0, as in glucose, however many Fe(III)-reducers prefer more oxidized organic acids such as lactate, which would require less Fe(III) reduction to produce CO₂.

[30] The high concentrations of Fe(III) in soil pore water were surprising for these soils, which were only moderately acidic (pH ~5.0). At moderate pH and under aerobic conditions, Fe(III) rapidly forms insoluble oxides, and so soluble forms of Fe(III) in mineral soils are generally assumed to be in the nM range [Zehnder and Stumm, 1988]. We are unaware of reports of such high soluble Fe(III) concentration in organic soils, though several studies have reported similarly high Fe(II) concentrations in fens and wetland sediments [Knorr and Blodau, 2009; Roden and Wetzel, 1996; Todorova et al., 2005]. Additionally, concentrations of Fe(III) comparable to our study have been occasionally observed in mineral soils, where the high concentrations were attributed to microbially produced siderophores [Ammari and Mengel, 2006]. The soluble Fe(III) concentrations in the range of hundreds of μM and the intercorrelations among DOC, UV absorbance, and Fe(III) in soil water imply the abundance of organic chelating molecules. Bacteria are known to produce a wide range of iron-chelating compounds that can greatly increase the solubility of Fe [Nevin and Lovley, 2002; Wandersman and Delepelaire, 2004], and carboxyl groups of humic substances can also complex Fe, increasing its solubility [Rakshit et al., 2009]. In most environments in which Fe(III) reduction has been

studied, it is insoluble Fe oxides that serve as terminal e⁻ acceptors. In these cases, e⁻ transfer is mediated by soluble carriers such as naturally occurring humic substances [Nevin and Lovley, 2002], microbially produced mediators such as phenazines, riboflavin, and melanin [Turick et al., 2002], and by direct cell-mineral contact [Reguera et al., 2005; Stams et al., 2006]. In the present study, there appears to be an ample supply of soluble Fe(III), presumably solubilized from the abundant forms of reducible Fe oxides found along the entire soil profile. Furthermore, addition of crystalline Fe(III) oxide did not stimulate respiration, while addition of soluble forms of Fe(III) did (though the form of Fe oxide used in this experiment was relatively stable, and so this remains to be tested with more easily reducible forms of Fe such as ferrihydrite). The preference for soluble Fe(III) is consistent with the observation that the most abundant bacterial 16S rRNA sequence was closely related to *R. ferrireducens*, which is able to reduce the soluble, chelated form, Fe(III)-nitrotriacetic acid, but not poorly crystalline Fe oxide [Finneran et al., 2003]. Interestingly, *R. ferrireducens* as originally described from sediment in Oyster Bay, VA, was capable of growth at 4°C, implying that its close relatives in this Arctic peat soil may be quite physiologically similar.

[31] Despite the prevalence of soluble Fe(III) in this ecosystem, there was also evidence that humic substances play a major role in anaerobic respiration: the addition of the quinone-containing humic acid analog AQDS stimulated respiration in laboratory incubations. The ability to reduce quinones is widespread among bacteria [Stams et al., 2006]. Our study site, with its humic-rich, anaerobic peat and its low levels of other alternative e⁻ acceptors such as nitrate or sulfate should favor this respiratory pathway, as was found in a study of a northern peat bog [Heitmann et al., 2007]. Quinone-like functional groups were found to be abundant in terrestrial-derived DOM from Toolik Lake, Alaska [Cory and McKnight, 2005; Fimmen et al., 2007]. It is likely that the redox cycles of Fe and humic substances are intimately connected in this soil. The addition of soluble Fe(III) to the soil stimulated respiration and lead to an overall drop in redox potential, implying that the increase in soluble Fe facilitated e⁻ transfer from energy sources (such as sugars and organic acids) to the soil matrix. Humic substances can serve both as e⁻ acceptors and donors for Fe and for microbial respiration [Heitmann et al., 2007; Knorr and Blodau, 2009; Lovley et al., 1996; Lovley et al., 1999; Peretyazhko and Sposito, 2006]. It is therefore possible that chelated Fe serves as an e⁻ shuttle between microbes and insoluble humics. This is the reverse of the frequently described case, in which soluble organic mediators transfer e⁻ between microbes and insoluble Fe(III) oxides [Nevin and Lovley, 2002; Stams et al., 2006]. Rapid cycling between Fe(II) and Fe(III) was observed in laboratory and field incubations, as well as in seasonal patterns in soil pore water. Also, a number of bacterial 16S rRNA gene sequences closely related to Fe-oxidizing bacteria were observed. This suggests that Fe oxidation could also be catalyzed microbiologically, in addition to abiotic reactions with humic substances or molecular oxygen.

[32] The dynamic nature of Fe cycling and the complex and heterogeneous nature of humic materials in these peat soils render it difficult to trace the e⁻ flow to a definitive

terminal e⁻ acceptor. However, the balance of evidence shows that Fe(III) and humic substances are important to respiration in peat soils of DTLB. Given this and the frequently reported negative relationship between Fe(III) and methane production, Fe(III) and humic reduction could have a significant influence on CO₂ and CH₄ fluxes from Arctic peat soils.

[33] **Acknowledgments.** The authors wish to thank numerous individuals who assisted in this work: Bethany Allen, Brian Brigham, Francis Bozzolo, Steve Hastings, Chun-Ta Lai, Marguerite Mauritz, Kyoko Nakamura, Freddy Ruiz, Yanfei Tang, and Joseph Verfaillie. Two anonymous reviewers provided highly detailed and helpful suggestions. This work was supported in part by NSF grants ARC-0808604 to D.A.L. and OPP-0421588 to W.C.O.

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